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(54) Method of staining, and detecting and counting bacteria

(57) A method of staining bacteria comprises: mixing a sample with an aqueous solution containing a cationic surfactant to accelerate dye transmissivity of bacteria at pH 2.0 to 4.5; and staining the bacteria for a predetermined period with a dye. A method of detecting bacteria comprises the following steps of: (1) mixing a sample with an aqueous solution containing a cationic surfactant and staining the bacteria with a dye by a method as described above, (2) introducing the thus treated sample into a detecting part of a flow cytometer and irradiating cells of the stained bacteria one by one with light to measure scattered light and fluorescent light emitted from each of the cells; and (3) discriminating the bacteria from other components in accordance with an intensity of a scattered light signal and an intensity of a fluorescent light signal or a pulse width reflecting the length of particles to count the bacteria.

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Description

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] The present invention relates to a method of staining, and detecting and counting bacteria in clinical samples, in particular, bacteria in urine samples.

Related Art

[0002] The number of bacteria in urine is an important parameter in clinical diagnosis to judge the presence of infection. In general, the presence of bacteria of 10^5 or more/ml in urine is recognized as a criterion of positive urinary tract infection. If urine contains bacteria of 10^3 or more/ml, it is diagnosed as contaminated urine (normal bacteria flora), i.e., negative urinary tract infection. If bacteria of about 10^4/ml is observed, the diagnosis is reserved but the sample is often re-examined.

[0003] Conventionally, observation of bacteria in urine has been performed by microscopic examination of Gram stained bacteria, unstained bacteria without Gram staining treatment or fluorescence-stained bacteria.

[0004] Urine often contains contaminants such as mucus threads, crystals, amorphous salts and cell fragments that are clinically insignificant. These substances hinder the measurement of significant particles (in particular bacteria) so that it has been difficult to accurately count the number of bacteria. Actually, there has been no method of counting bacteria of about 10^4/ml, accurately.

[0005] In the case of Gram stain, bacteria and contaminants are stained simultaneously so that counting loss of bacteria of a small number occurs frequently in the microscopic examination. Further, Gram stain includes a number of staining steps and takes time (about 15 minutes) so that working efficiency is poor.

[0006] The microscopic examination of bacteria without staining treatment can be carried out quickly, but it cannot discriminate bacteria particularly when coccus contaminants are contained.

[0007] The microscopic examination of fluorescence-stained bacteria shows better detectability than the above-mentioned two methods. However, there has not been established how to eliminate other contaminants than bacteria and to stain the bacteria quickly.

[0008] Agar medium method, which is a standard method, requires 16 hours or more to determine the bacteria number, so that it cannot be regarded as a quick method.

[0009] USP 4,622,298 and Japanese Unexamined Patent Publication No. Hei 9 (1997)-119926 each proposes a method of detecting bacteria in a fluorescence-stained urine sample with a flow cytometer. However, in the above references, examination of a sample including contaminants is not conducted.

[0010] Japanese Unexamined Patent Publication No. Hei 9 (1997)-329596 describes an analysis of solid components in urine which are difficult to be discriminated from each other. In this method, a urine sample is treated with an aqueous solution containing a surfactant and examined with a flow cytometer. However, it does not describe a method of discrimination between bacteria and contaminants.

SUMMARY OF THE INVENTION

[0011] An object of the present invention is to provide a method of staining, and detecting and counting bacteria which allows quick and efficient detection of bacteria without performing cultivation even if a sample contains contaminants.

[0012] The present invention provides a method of staining bacteria comprising: mixing a sample with an aqueous solution containing a cationic surfactant to accelerate dye transmissivity of bacteria at pH 2.0 to 4.5; and staining the bacteria for a predetermined period with a dye.

[0013] Further, the present invention provides a method of detecting and counting bacteria comprising the following steps of:

(1) mixing a sample with an aqueous solution containing a cationic surfactant and staining the bacteria with a dye by a method as described in the above;
(2) introducing the thus treated sample into a detecting part of a flow cytometer and irradiating cells of the stained bacteria one by one with light to measure scattered light and fluorescent light emitted from each of the cells; and
(3) discriminating the bacteria from other components in accordance with an intensity of a scattered light signal and an intensity of a fluorescent light signal or a pulse width reflecting the length of particles to count the number of the bacteria.

BRIEF DESCRIPTION OF THE DRAWINGS

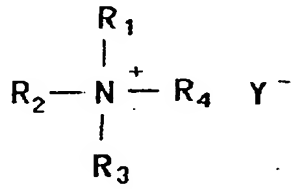
[0014]

Fig. 1 is a scattergram of a fluorescent light intensity - a forward scattered light intensity obtained in the case where cultivated E. coli was examined in Example 1 of the present invention;
Fig. 2 is a scattergram of a fluorescent light intensity - a forward scattered light intensity obtained in the case where various cultivated bacteria are examined in Example 2 of the present invention;
Fig. 3 is a graph illustrating the results of a dilution linearity test performed in Example 3 of the present invention;
Fig. 4 is a graph illustrating correlation between measurement results according to the present invention and those of a CLED medium cultivation obtained in Example 4 of the present invention;
Fig. 5 is a scattergram illustrating measurement results of Gram-negative bacillus obtained in Example 5 of the present invention;
Fig. 6 is a scattergram illustrating measurement results of Gram-positive bacillus obtained in Example 6 of the present invention; and
Fig. 7 is a view illustrating the outline of the method of detecting bacteria according to the present invention.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0015] In the present invention, the sample is not particularly limited as long as it is a sample to be examined for the presence or absence of bacteria and to count a number of bacteria if the sample contains bacteria. Bacteria referred herein include bacteria observed in a urine sample such as E.coli, Klebsiella sp., as well as Staphyrococcus sp., Pseudomonas sp., Serratia sp., Enterobacter sp., Enterococcus sp., Streptpococcus sp. and Citrobacter sp. For example, the sample may be a clinical sample such as urine, blood, spinal fluid or the like. The sample may be diluted with purified water or the like two or more times, preferably 4 to 15 times, more preferabley 5 to 10 times. The present invention is particularly effective for a urine sample.

[0016] No particular limitation is given to the cationic surfactant, but preferably used is a quarternary ammonium salt represented by the following formula:



wherein R_1 is a C_{8-18} alkyl group; R_2, R_3 and R_4, the same or different, are a C_{1-3} alkyl group or benzyl group; Y^- is a halogen ion.

[0017] The C_{1-3} alkyl group may be methyl, ethyl, propyl and the like. The C_{8-18} alkyl group may be octyl, decyl, dodecyl, tetradecyl and the like.

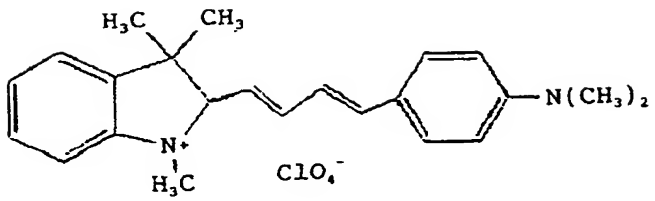
[0018] For example, suitably used are decyl trimethyl ammonium salt, dodecyl trimethyl ammonium salt, tetradecyl trimethyl ammonium salt, hexadecyl trimethyl ammonium salt, octadecyl trimethyl ammonium salt and the like. Appropriate use amount thereof may be 10 to 30000 mg/l, preferably 100 to 3000 mg/l in the sample (final concentration).

[0019] Addition of the cationic surfactant to the bacteria-containing sample damages cell membrane of bacteria so that a dye easily enters the cell. As a result, content of the bacteria cell and the dye are efficiently bonded to each other so that bacteria are stained well, which facilitates the discrimination of bacteria from contaminants. Further, mucus threads, erythrocytes and cell fragments are lysed or shrunk, which reduces influence thereof on the bacteria detection.

[0020] The dye is not particularly limited as long as it can stain bacteria. Where a urine sample is examined, a dye capable of staining bacteria under an acidic state is preferably used. The concentration thereof may suitably be determined depending on the kind of dye, for example, in the range of 0.1 to 100 ppm (final concentration). In view of bacteria detectability, a fluorescent dye which is at least bonded to one of components constituting bacteria and emits fluorescent light is advantageously used. From this point of view, polymethine dyes are preferable. For example, the following dyes (1) to (11) are used:

(1) Thiazole Orange;

(2);



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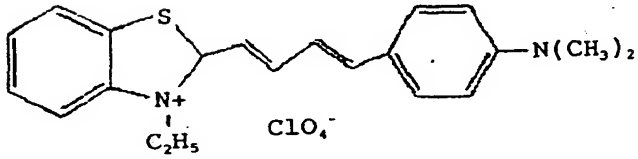

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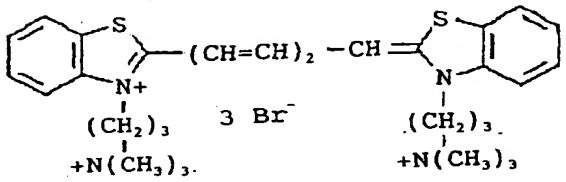

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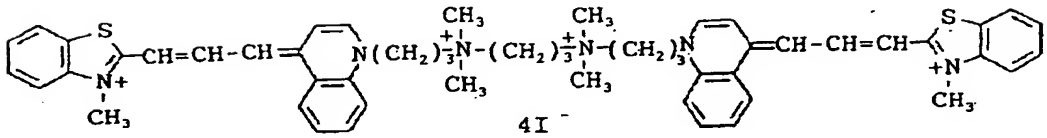

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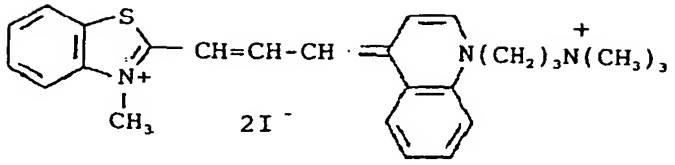

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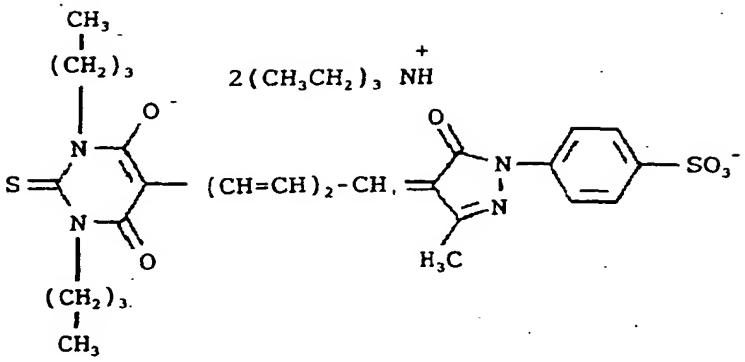

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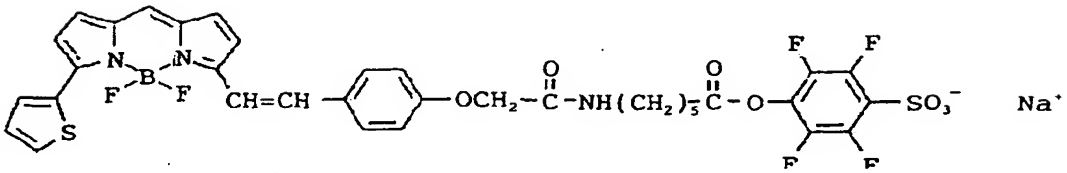

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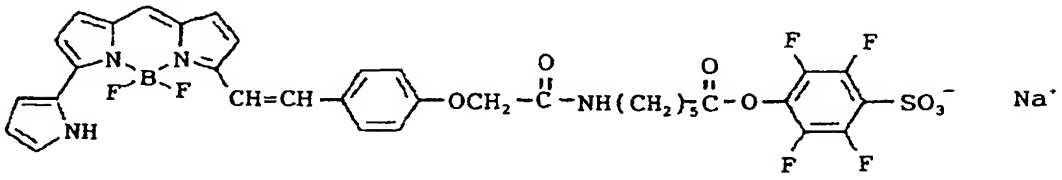
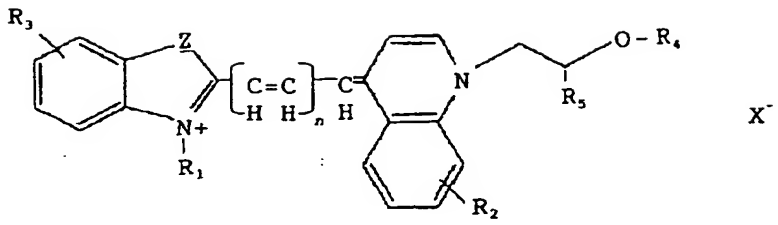
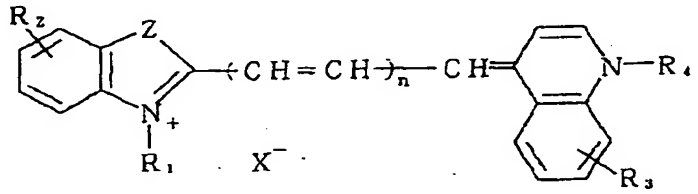


(10) a compound represented by the following general formula:



wherein R_1 is a hydrogen atom or a C_{1-3} alkyl group; R_2 and R_3 are a hydrogen atom, a C_{1-3} alkyl group or a C_{1-3} alkoxy group; R_4 is a hydrogen atom, an acyl group or a C_{1-3} alkyl group; R_5 is a hydrogen atom or a C_{1-3} alkyl group which may be substituted; Z is a sulfur atom, an oxygen atom or a carbon atom substituted with a C_{1-3} alkyl group; n is 1 or 2; X^- is an anion; and

(11) a compound represented by the following general formula:

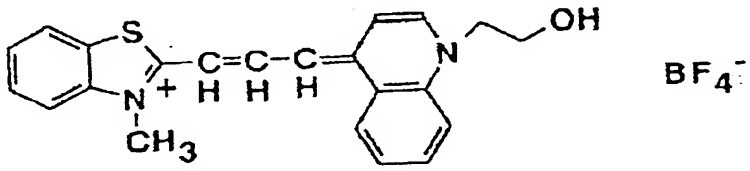


wherein R_1 is a hydrogen atom or a C_{1-18} alkyl group; R_2 and R_3 are a hydrogen atom, a C_{1-3} alkyl group or a C_{1-3} alkoxy group; R_4 is a hydrogen atom, an acyl group or a C_{1-18} alkyl group; Z is sulfur, oxygen or a carbon atom substituted with a C_{1-3} alkyl group; n is 0, 1 or 2; X^- is an anion.

[0021] The C_{1-3} alkyl group may be methyl, ethyl, propyl and the like. The C_{1-18} alkyl group may be methyl, ethyl, propyl, octyl, decyl, dodecyl, tetradecyl and the like. The a C_{1-3} alkoxy group may be methoxy, ethoxy, propoxy and the like. Substituents to the C_{1-3} alkyl group may be a hydroxyl group, a halogen atom and the like.

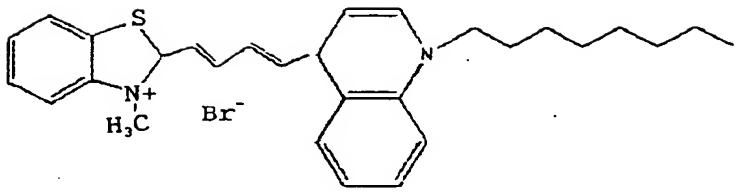
[0022] Among the above-mentioned dyes, (1) is commercially available. (2) and (3) are supplied by Nippon Photosensitive Dye Laboratory Ltd., and (5) to (9) are supplied by Molecular Probes, Inc. Manufacturing methods of (10) and (11) are described in Japanese Unexamined Patent Publications Nos. Hei 9(1997)-104683 and Hei 10(1998)-319010, respectively.

[0023] Among the dyes (10), a dye represented by the formula:



is particularly suitable.

[0024] Further, among the dyes (11), a dye represented by the formula:



is particularly suitable.

[0025] In the present invention, pH at the staining step is not specifically limited as long as it allows the bacteria staining. Where a urine sample is stained at an acidic pH, preferably pH 2 to 4.5, more preferably pH 2 to 3, (1) bacteria is stained better than in a neutral or alkaline state and (2) nonspecific staining of mucus threads is prevented and the mucus threads is lysed to a certain extent. Thus, the acidic state is advantageous to the bacteria staining.

[0026] A buffer of pKa 1 to 5.5 is used to maintain the acidic state. The buffer is not particularly limited, but an acid or a compound capable of maintaining pH 2.0-3.0 may be used. As the buffer, it may be utilized one or more kinds of compounds selected from the group comprising of: citric acid or its salts, phosphoric acid or its salts, phthalic acid or its salts, succinic acid or its salts, lactic acid or its salts, ε-aminocaproic acid or its salts, fumaric acid or its salts, β-alanine, glycine and the like. The salts described above include alkali or alkaline earth salts. Suitable examples thereof is at least one selected from the group consisting of: citric acid-NaOH, citric acid -sodium citrate, potassium dihydrogen phosphate-disodium hydrogen phosphate, potassium dihydrogen phosphate-NaOH, citric acid-disodium hydrogen phosphate, potassium hydrogen phthalate-NaOH, succinic acid-NaOH, lactic acid-NaOH, ε-aminocaproic acid-HCl, fumaric acid-HCl, β-alanine-NaOH, glycine-NaOH and the like. Appropriate use amount thereof is such that the above-mentioned pH range is maintained, preferably about 10 to 500 mM in the sample.

[0027] Further, where a urine sample is examined, the staining is carried out by further utilizing an inorganic salt of either sulfate or nitrate. This is preferable since fluorescent dye transmissivity of bacteria is enhanced and nonspecific staining of contaminants is prevented. The inorganic salt may be used in a concentration of about 10 to 500 mM, preferably about 50 to 200 mM in the sample.

[0028] In the present invention, the working a dye on a sample (staining) may be carried out by mixing the sample, one by one or simultaneously, an aqueous solution containing the cationic surfactant and a solution containing the dye. The dye may be contained in the aqueous solution containing the cationic surfactant. However, where the dye to be utilized is unstable in the aqueous solution, it may be dissolved in a water-soluble organic solvent such as methanol, ethanol or ethylene glycol and then mixed for use with the aqueous solution containing the substance capable of reducing nitrite ions and/or the cationic surfactant. This improves storage stability of the dye.

[0029] Temperature and time for the staining are not particularly limited, but the staining may be performed at about 15 to 50°C for about 20 minutes or less, preferably about 15 minutes or less, more preferably about 15 minutes immediately after the mixing.

[0030] The sample stained by the method of the present invention may be observed with a microscope or an imaging apparatus to detect bacteria. Alternatively, bacteria can be detected and counted by using a flow cytometer with high accuracy. The flow cytometer used herein may be a commercially available apparatus generally utilized in the art.

[0031] That is, the method of detecting and counting bacteria according to the present invention is carried out by the steps of:

(1) mixing a sample with an aqueous solution containing a cationic surfactant and staining the bacteria with a dye by a method as described above,

(2) introducing the thus treated sample into a detecting part of a flow cytometer and irradiating cells of the stained bacteria one by one with light to measure scattered light and fluorescent light emitted from each of the cells; and

(3) discriminating the bacteria from other components in accordance with an intensity of a scattered light signal and an intensity of a fluorescent light signal or a pulse width reflecting the length of particles to count the bacteria.

[0032] The method of step (1) may be performed as described above, e.g., by mixing a sample with an aqueous solution containing a cationic surfactant to accelerate dye transmissivity of bacteria and then (or simultaneously) staining the sample for a certain period with a dye.

[0033] Discrimination of bacteria from other components and counting of bacteria are carried out in accordance with combination of signals obtained by using a flow cytometer. Example of the combination includes, for example, a forward scattered light intensity and a forward scattered light pulse width, a forward scattered light intensity and a fluorescent

light intensity, a forward scattered light pulse width and a fluorescent light intensity, and the like. In a suitable manner, for example, firstly, a scattergram is formed from the combination of the forward scattered light intensity and the forward scattered light pulse width, and then gating is performed to a mass including bacteria specified on the scattergram to separate mucus threads, mainly. Further, another scattergram is formed from the forward scattered light intensity and the fluorescent light intensity of the gated mass to separate bacteria from other components (crystals, cell fragments and the like) based on the difference in the fluorescent light intensity. The outline of the method is shown in Fig. 7. Where the sample contains bacteria only, a scattergram is formed from the forward scattered light intensity and the fluorescent light intensity to count them.

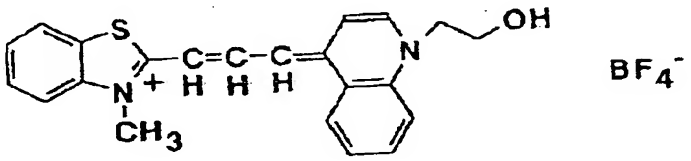
Examples

[0034] Hereinafter, preferred examples of the method of staining and detecting bacteria according to the present invention are described, but the present invention is not limited thereto.

Example 1

[0035]

Reagent Composition	
(Diluent)	
Citric acid	100 mM
NaOH	up to pH 4.1
Tetradecyl trimethyl ammonium bromide	0.1 %(w/v)
(Staining solution)	
Dye A (of the above-mentioned formula)	40 ppm (in ethylene glycol)



[0036] To 100 µl of a sample containing cultivated E. coli, 1000 µl of the above-mentioned diluent was added and the staining solution was added so that the final concentration of the dye A would be 1 ppm. Staining was carried out at 40°C for 30 seconds and then scattered light and fluorescent light were measured by the flow cytometer provided with a red semiconductor laser as a light source (amount of examined urine: 7.8 µl). As a control, measurement was performed using a reagent in which tetradecyl trimethyl ammonium bromide was not contained. The results are shown in Fig. 1.

[0037] In the case where the reagent without tetradecyl trimethyl ammonium bromide was used (left scattergram), the fluorescent light intensity was 30 ch or less, i.e., few E. coli was stained. In contrast, where the reagent contained 0.1% (w/v) tetradecyl trimethyl ammonium bromide (right scattergram), a mass of E. coli was distributed around the fluorescent light intensity of 50 ch or more. Thus, it was observed that dye transmissivity was enhanced.

Example 2

[0038]

Reagent Composition	
(Diluent)	
Citric acid	100 mM (pH 2.5)
Tetradecyl trimethyl ammonium bromide	0.1 %(w/v)
Sodium sulfate	90 mM

(Staining solution)

Same as Example 1

[0039] With the above-mentioned reagent, samples each containing cultivated bacteria (E.coli, S.aureus, K.pneumoniae, C.freundii, E.faecalis) were examined in the same manner as in Example 1. The results are shown in Fig. 2.
[0040] Where the reagent without tetradecyl trimethyl ammonium bromide was used, the fluorescent light intensity in the samples was weak, i.e., few bacteria were stained. However, the addition of tetradecyl trimethyl ammonium bromide caused increase in the fluorescent light intensity and thus it was observed the bacteria were stained well. In comparison of E. coli measurement between the present Example in which sodium sulfate was further added and Example 1 in which sodium sulfate was not contained, it is shown that the fluorescent light intensity was more enhanced in the present Example, i.e., the addition of sodium sulfate was effective.

Example 3

Dilution linearity

[0041] E. coli was cultivated and diluted samples each diluted at dilution coefficient of 1, 10, 100, 1000, 10000 and 100000 were prepared to perform measurement by utilizing the reagent used in Example 2. Fig. 3 shows the results. The bacteria number was obtained by gating a mass including bacteria specified on the scattergram formed from the forward scattered light intensity and the fluorescent light intensity
[0042] As shown in Fig. 3, favorable linearity was obtained in the bacteria concentration range of 10^3 to 10^7/ml.

Example 4

Examination of urine sample

[0043] With the reagent used in Example 2, 62 urine samples were examined and consideration was given to the correlation between the results thereof and those obtained by cultivation on a CLED medium performed as a control.
[0044] For measurement of the bacteria number, a scattergram was formed from a combination of the forward scattered light intensity and the forward scattered light pulse width and then gating was performed to a mass including bacteria specified on the scattergram. Then, another scattergram was formed from a combination of the forward scattered light intensity and the fluorescent light intensity of the gated mass. A region of bacteria was specified from the difference in the fluorescent light intensity to count bacteria in the specified region. The results are shown in Fig. 4.
[0045] Favorable correlation with the results of the cultivation on the CLED medium. In Fig. 4, several points are observed along the vertical axis. This is because bacteria which hardly grow on medium (static bacteria; bacteria whose growth is hindered by agents or the like) and dead bacteria are also measured in the present invention, though the cultivation on the CLED medium detects living bacteria only.

Example 5

Examination of blood culture sample

[0046] Blood sample containing bacteria was cultivated in a blood culture bottle and the cultivated fluid was measured. Measurement results of Gram-negative bacillus (Pseudomonas sp.) are shown in Fig. 5 and those of Gram-positive coccus (Staphyrococcus sp.) are shown in Fig. 6. To count the bacteria number, a scattergram was formed

from a combination of the forward scattered light intensity and the fluorescent light intensity and gating was performed to a mass including bacteria was specified on the scattergram. KOBA 10 grid (HYCOR BIOMEDICAL INC.) was used as a control to perform visual observation. In the method of the present invention, Gram-negative bacillus was measured 5.2×10^5/ml and Gram-positive coccus was measured 2.3×10^4/ml. In the visual observation, they were measured 2.9×10^5/ml and 8.8×10^3/ml, respectively.

[0047] According to the method of staining bacteria of the present invention, since bacteria are stained in an aqueous state, dry fixation such as Gram staining is not necessarily required. Therefore, staining period can be remarkably reduced and thus a sample for measurement can be prepared in a short time including the staining step.

[0048] Since the staining according to the present invention can easily be performed by merely mixing the sample and the reagent, skill required in Gram staining is eliminated. Further, the staining step can be easily carried out, which facilitates the automation through the staining step to the measurement step (such as flow cytometory and image analysis).

[0049] According to the method of detecting bacteria of the present invention, bacteria can be counted with high accuracy without being affected by the contaminants. Specifically, bacteria of 10^4/ml can be counted.

[0050] Further, bacteria whose growth is difficult on medium (bacteriostatic samples) can also be counted reliably.

Claims

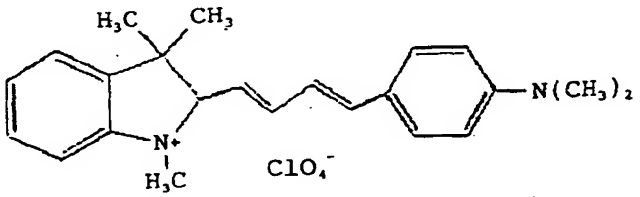
1. A method of staining bacteria comprising:

   mixing a sample with an aqueous solution containing a cationic surfactant to accelerate dye transmissivity of bacteria at pH 2.0 to 4.5; and
   staining the bacteria for a predetermined period with a dye.

2. A method according to claim 1, wherein the dye is a fluorescent dye which is at least bonded to one of components constituting the bacteria.

3. A method according to claim 1, wherein the dye is at least one selected from the following group consisting of:

   (1) Thiazole Orange;
   (2)



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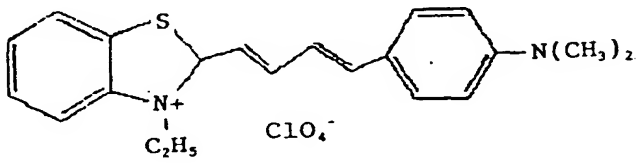

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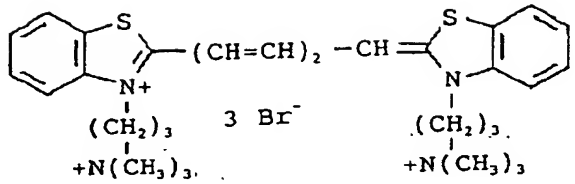

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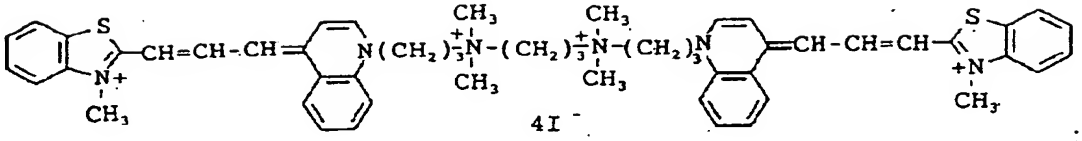

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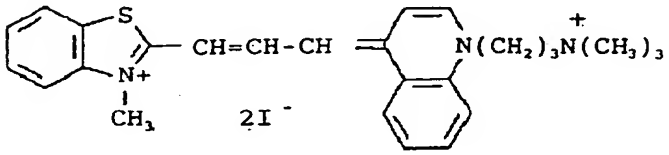
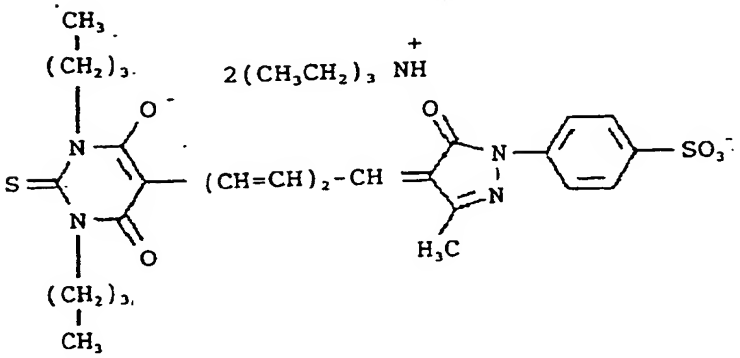
(6)



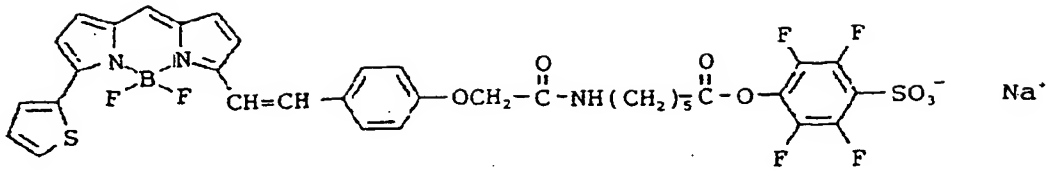
;

(7)



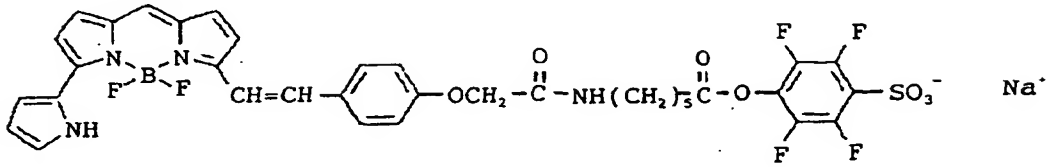
;

(8)



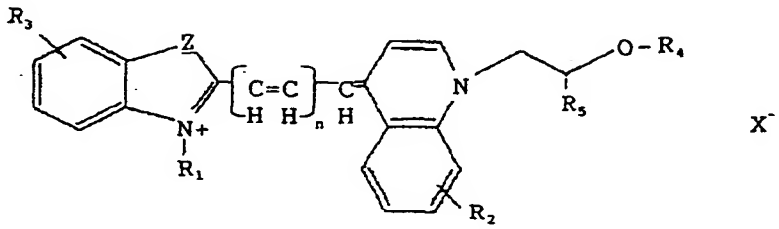
;

(9)



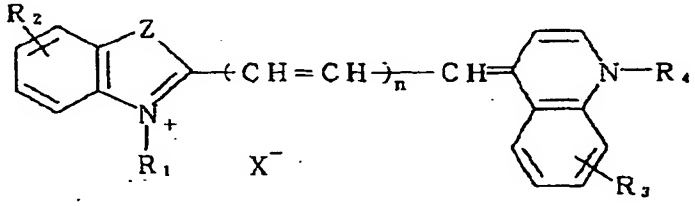
;

(10) a compound represented by the following general formula:



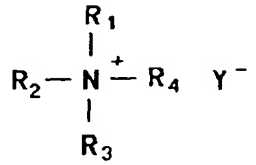
wherein R_1 is a hydrogen atom or a C_{1-3} alkyl group; R_2 and R_3 are a hydrogen atom, a C_{1-3} alkyl group or a C_{1-3} alkoxy group; R_4 is a hydrogen atom, an acyl group or a C_{1-3} alkyl group; R_5 is a hydrogen atom or a C_{1-3} alkyl group which may be substituted; Z is a sulfur atom, an oxygen atom or a carbon atom substituted with a C_{1-3} alkyl group; n is 1 or 2; X^- is an anion; and

(11) a compound represented by the following general formula:



wherein R_1 is a hydrogen atom or a C_{1-18} alkyl group; R_2 and R_3 are a hydrogen atom, a C_{1-3} alkyl group or a C_{1-3} alkoxy group; R_4 is a hydrogen atom, an acyl group or a C_{1-18} alkyl group; Z is sulfur, oxygen or carbon having a C_{1-3} alkyl group; n is 0, 1 or 2; X^- is an anion.

4. A method according to claim 1, wherein the cationic surfactant is a quaternary ammonium salt represented by the following formula:



wherein R_1 is a C_{8-18} alkyl group; R_2, R_3 and R_4, the same or different, are a C_{1-3} alkyl group or a C_{1-3} benzyl group; Y^- is a halogen ion.

5. A method according to claim 4, wherein the quaternary ammonium salt is at least one selected from the group consisting of: decyl trimethyl ammonium salt, dodecyl trimethyl ammonium salt, tetradecyl trimethyl ammonium salt, hexadecyl trimethyl ammonium salt and octadecyl trimethyl ammonium salt.

6. A method according to claim 1, wherein a buffer of pKa 1 to 5.5 is used to maintain pH.

7. A method according to claim 10, wherein the buffer is at least one selected from the group consisting of: citric acid-NaOH, citric acid -sodium citrate, potassium dihydrogen phosphate-disodium hydrogen phosphate, potassium dihydrogen phosphate-NaOH, citric acid-disodium hydrogen phosphate, potassium hydrogen phthalate-NaOH, succinic acid-NaOH, lactic acid-NaOH, ε-aminocaproic acid-HCl, fumaric acid-HCl, β-alanine-NaOH and glycine-NaOH.

8. A method according to claim 1, wherein the working is carried out in the existence with an inorganic salt of either sulfate or nitrate.

9. A method according to claim 1, wherein the dye is worked at 0.1 to 100 ppm in the sample.

10. A method according to claim 1, wherein the cationic surfactant exists at 10 to 30000 mg/l in the sample.

11. A method according to claim 7, wherein the acid or the compound maintaining an acidic pH exists at 10 to 500 mM in the sample.

12. A method of detecting and counting bacteria comprising the following steps of:

(1) mixing a sample with an aqueous solution containing a cationic surfactant and staining the bacteria with a dye by a method as described in any one of claims 1 to 11;
(2) introducing the thus treated sample into a detecting part of a flow cytometer and irradiating cells of the stained bacteria one by one with light to measure scattered light and fluorescent light emitted from each of the cells; and
(3) discriminating the bacteria from other components in accordance with an intensity of a scattered light signal and an intensity of a fluorescent light signal or a pulse width reflecting the length of particles to count the number of the bacteria.

13. A method according to claim 12, wherein the step (3) of discriminating and counting the bacteria is carried out in accordance with at least one selected from the following combinations of:

(i) a forward scattered light intensity and a forward scattered light pulse width;
(ii) a forward scattered light intensity and a fluorescent light intensity; and
(iii) a forward scattered light pulse width and a fluorescent light intensity.

FIG. 1

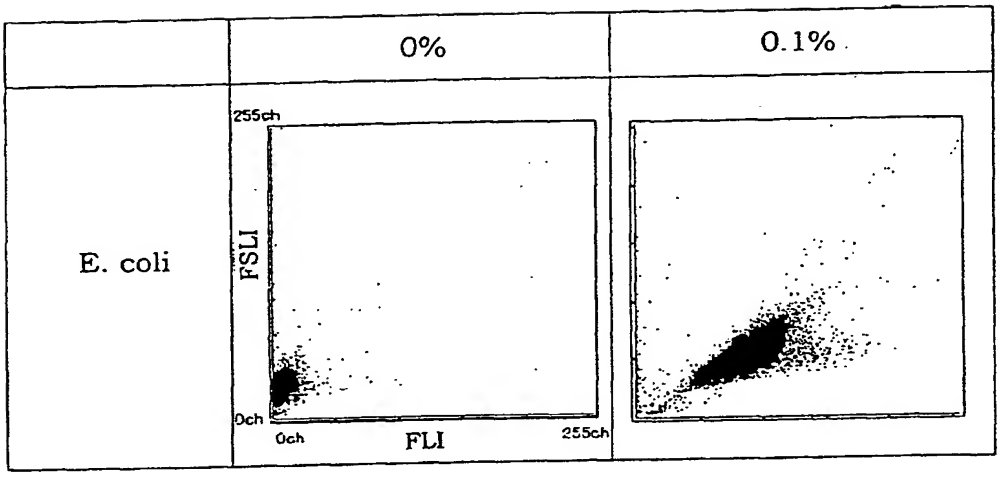


FIG. 2

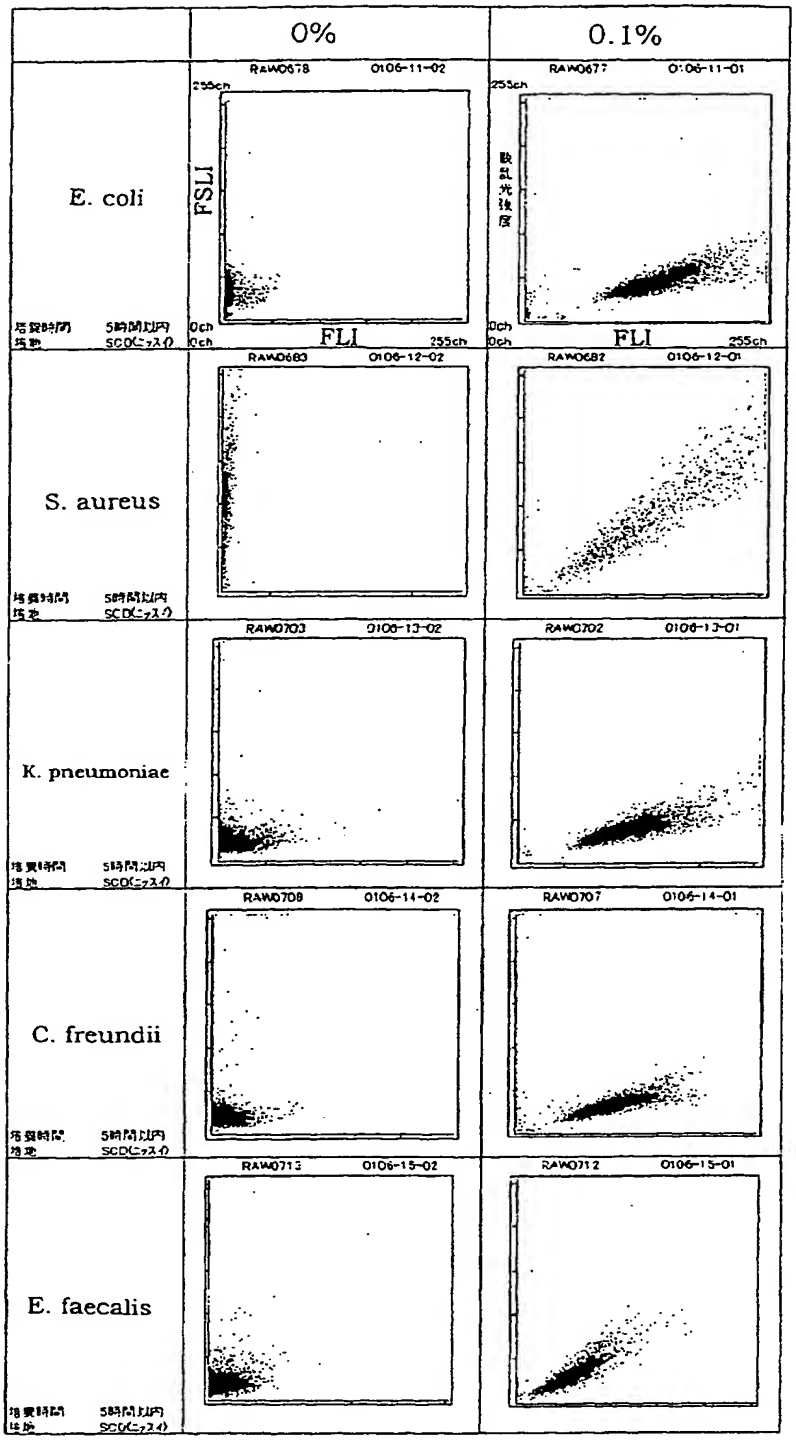

0%
0.1%
E. coli
S. aureus
K. pneumoniae
C. freundii
E. faecalis
FSLI
FLI
255ch
0ch
RAW0678
0106-11-02
RAW0677
0106-11-01
RAW0683
0106-12-02
RAW0682
0106-12-01
RAW0703
0106-13-02
RAW0702
0106-13-01
RAW0708
0106-14-02
RAW0707
0106-14-01
RAW0713
0106-15-02
RAW0712
0106-15-01

FIG. 3

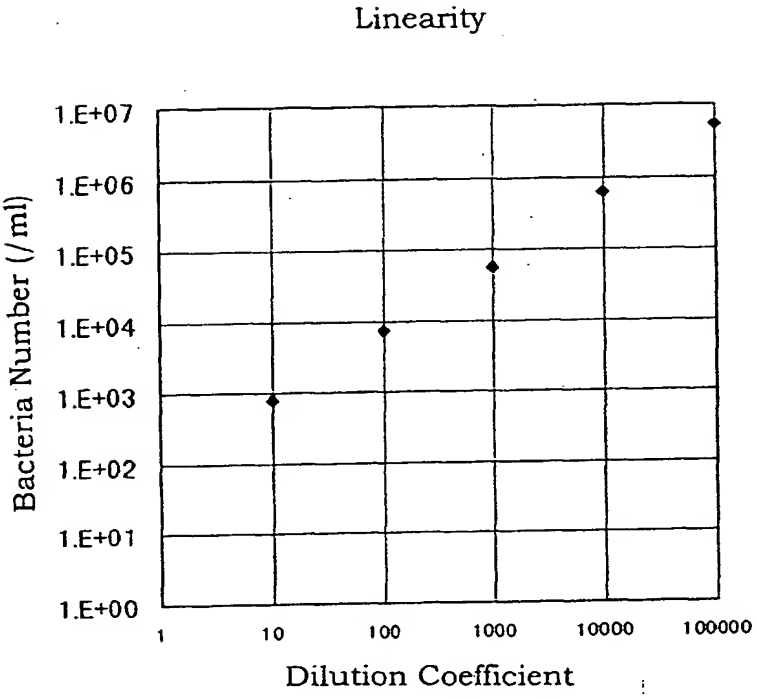

Linearity
Bacteria Number (/ml)
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
1
10
100
1000
10000
100000
Dilution Coefficient

FIG. 4

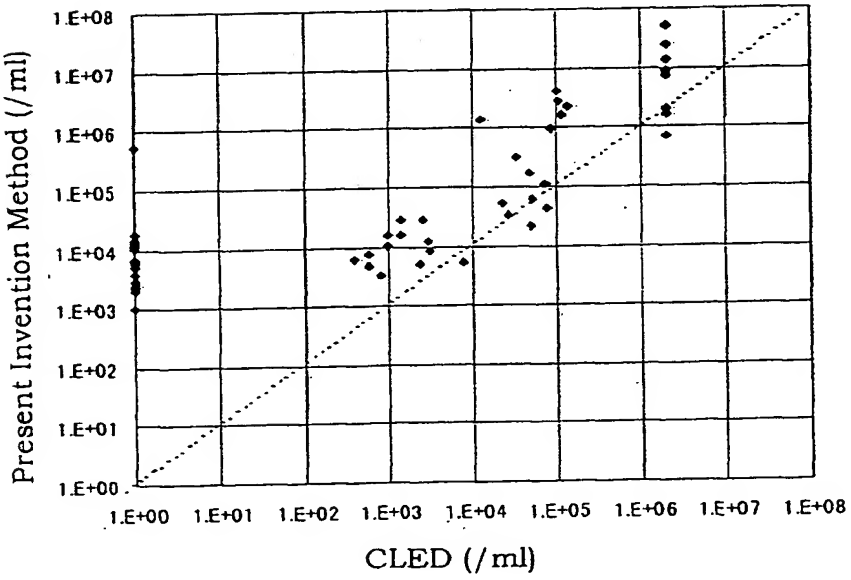

Present Invention Method (/ml)
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
1.E+00
1.E+01
1.E+02
1.E+03
1.E+04
1.E+05
1.E+06
1.E+07
1.E+08
CLED (/ml)

FIG. 5

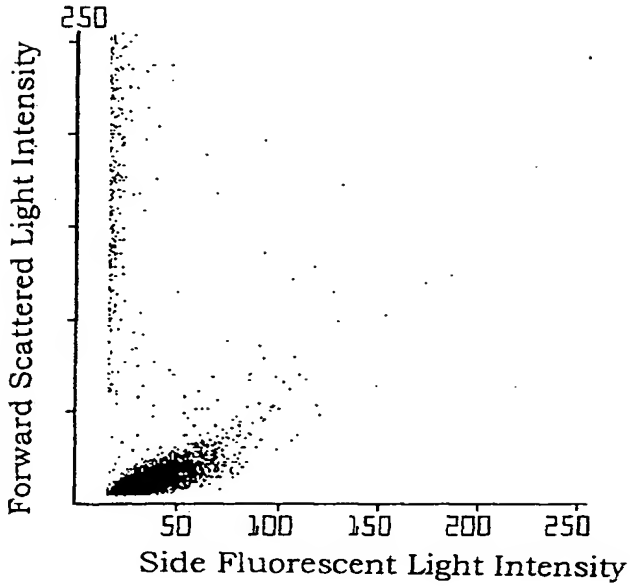

250
Forward Scattered Light Intensity
50
100
150
200
250
Side Fluorescent Light Intensity

FIG. 6

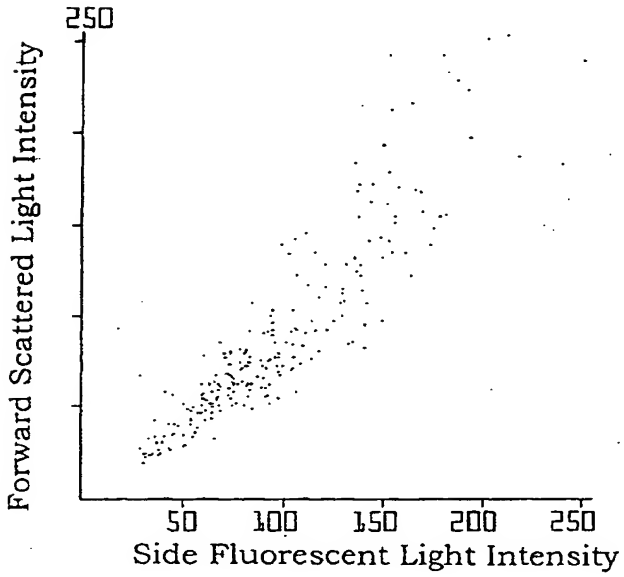
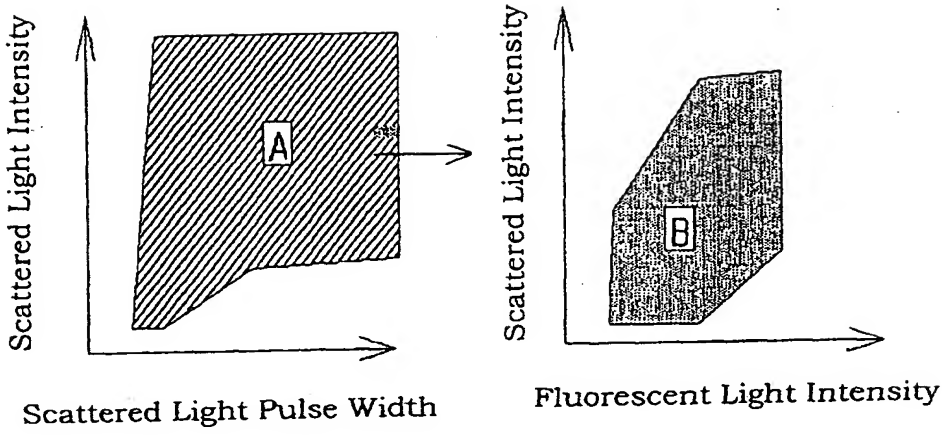

250
Forward Scattered Light Intensity
50
100
150
200
250
Side Fluorescent Light Intensity

FIG. 7


Scattered Light Intensity
A
Scattered Light Pulse Width
Scattered Light Intensity
B
Fluorescent Light Intensity